NATURAL VARIATION IN ROOTING ABILITY OF WESTERN PROVENANCES OF SHORTLEAF PINE

By

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OF WESTERN PROVENANCES OF

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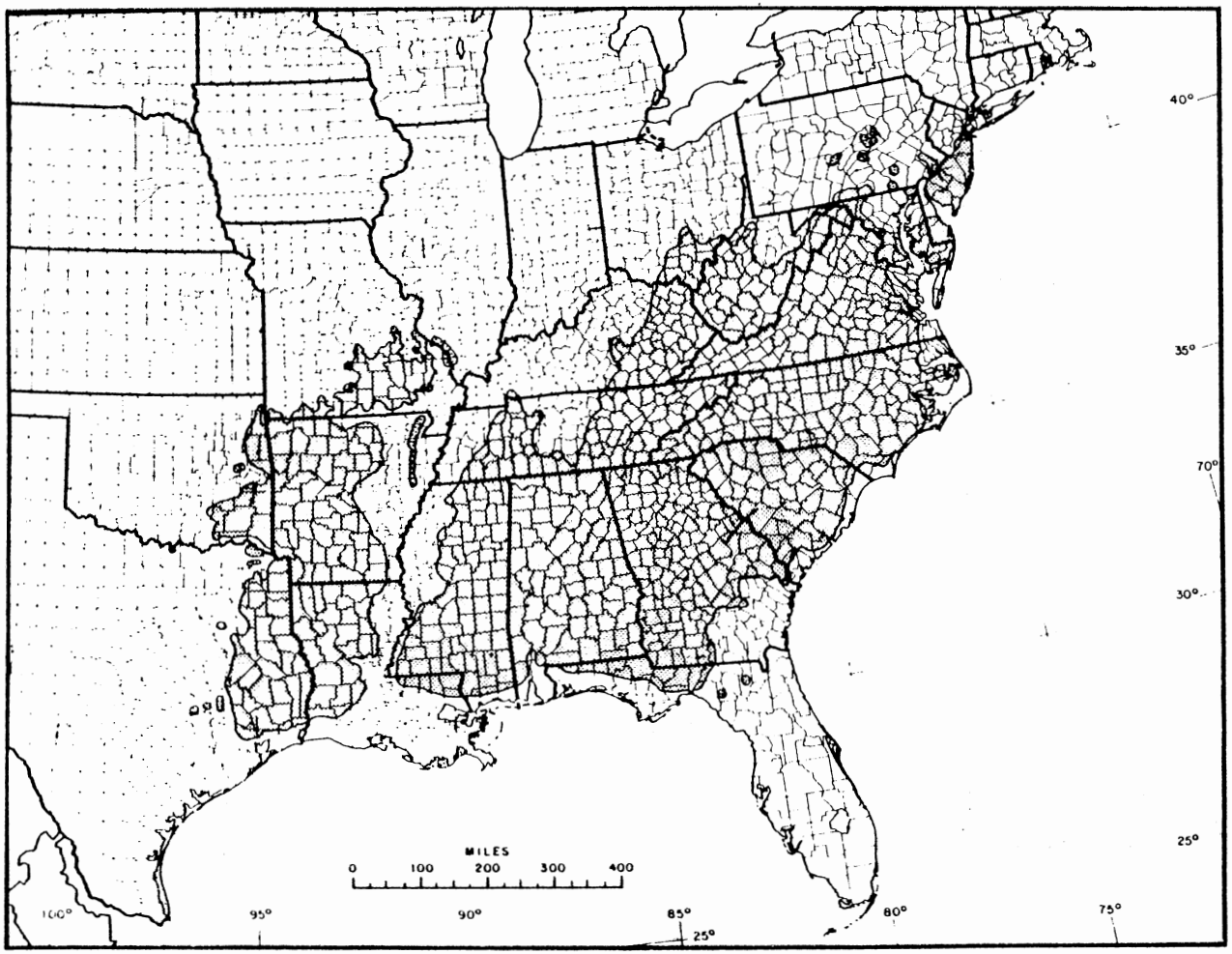
CHAPTER I

INTRODUCTION

Shortleaf Pine (Pinus echinata Mill.) is an economically important species whose geographic range includes 24 states and extends from eastern Oklahoma and Texas to New Jersey (Figure 1). The species is distributed altitudinally from sea level to over 3,000 feet in portions of the Appalachian Mountains. Although shortleaf pine seedlings can be produced at a comparable cost in relation to other southern pines, its somewhat slower growth as compared to loblolly (Pinus taeda L.) and slash pine (Pinus elliottii Engelm.), and its susceptibility to littleleaf disease have prevented extensive planting of this species in areas of Alabama eastward through Georgia (Dorman, 1976). In the northern part of its natural range, and in the eastern states (the Carolinas north to Pennsylvania and New Jersey), shortleaf pine remains an economically important commercial species. In areas of the central United States (north of the range of the other major southern pines) there has been a continuing interest in shortleaf pine as an introduced species (Dorman, 1976).

Loblolly pine has been the predominant reforestation species in southeastern Oklahoma and western Arkansas for the past ten years. However, shortleaf pine has maintained its status as the best species for well drained, moderately dry intermountain regions, indicating its ecological adaptability to less than optimum sites.



Source: Dorman, (1976, p.7)

Figure 1. Natural Range of Shortleaf Pine (Pinus echinata Mill.)

In view of the wide geographic and altitudinal distribution of shortleaf pine, it is understandable that the species possesses marked natural variation in many characteristics from one geographic region to the next. As with many wide ranging forest tree species, shortleaf pine appears to be composed of moderately distinct geographic ecotypes. Therefore, it is possible that some pattern of variation in rooting ability could exist.

During the past ten years interest in forest genetics has increased greatly, with the major interest centering around among family variability and its utilization. Effective selection for a plant character can be achieved only if genetic variation exists within the population of interest (Briggs and Knowles, 1976). Dorman (1976) stated that studies on racial variation will indicate whether inherent differences occur among groups of trees in various population centers. If there are inherent differences among populations and such variation provides one individual with an advantage over another in its ability to root and grow, that advantage should be used to its fullest extent in tree improvement programs.

Increased utilization of forests for traditional uses (watershed, wildlife habitat, firewood, wood products, and recreation) continues to place a heavy strain on these resources. As the demand increases, so do the pressures on the managers of these resources. The forest products industry has been faced with a continuing shrinkage of the land base available for production of raw material. One option available to the producer is to rely upon tree improvement research to produce faster growing, higher yielding trees. Libby (1976) described the procedure currently used in tree improvement:

> Classical tree improvement for forestry purposes has used selected superior trees gathered together in seed orchards to get the selected genes back into the forest in higher frequencies. The most common way to create a seed orchard is to graft scions from outstanding selected trees, onto vigorous, healthy growing rootstock, replicating each clone many times to achieve a large production of seeds (p. 28).

This author's research evolved from the current interest in using vegetative propagation as a tool for tree improvement. There is a need for rooting studies to evaluate the variability in rooting ability and in characteristics of the roots of vegetatively propagated clones to determine if, among other things, selection for certain characteristics of the roots can increase overall productivity.

If rooting ability and physical characteristics of the roots tend to be sufficiently heritable, clones that have proven to be compatable for grafting could be selected on the basis of their rooting ability and root structure, then vegetatively propagated to provide superior rootstock. In fruit trees it has been found that the unique structure and functioning of the understock of any given genotype has an influence on almost every characteristic of the composite plant, including growth rate, cold resistance, size and abundance of fruit, and overall vigor of the plant (Rogers and Beakbane, 1957). Libby (1975) stated that clonal testing of rooted cuttings might be used to identify individual trees with outstanding characteristics due to a particular non-additive combination of genes.

As vegetative propagation of commercial forest species becomes more efficient and productive, procedures for evaluating rooting ability will become more important in the role of tree improvement.

Dr. Ed Kerr (1977) (Management Branch, Southern Forest Experiment Station) stated that Dr. Hare's work to date (1977) with vegetative

propagation of pine could lead to the direct reforestation of some sites with pine by planting genetically superior cuttings rather than the conventional planting of seedlings. Libby (1976) pointed out that in the attempt by foresters to find more effective techniques for managing our timber resources, the genetic advancement possible using vegetative propagation in tree improvement and reforestation is an attraction worth consideration. Van Buijtenen et al. (1973) stated that genetic gain with vegetative propagation would be double that obtained from seedlings if the results currently obtained using rooted cuttings with other species, such as cottonwood (Populus deltoides Bartr.), are any indication of the advancement possible with pine.

Libby (1976) described the following orchard technique which was tested using Monterey pine (Pinus radiata D. Don) in Australia (Libby, Brown, and Fielding, 1972). A cutting orchard was devised to supply vegetative material for propagation. The cutting orchard was composed of small trees periodically trimmed to produce hedges. As new select genotypes became available, they were added to the cutting orchard and provided vegetative material in years that seed production was low. Libby (1976) states that if this technique was adopted, conventional seed orchards could be reduced to small, genetically diverse breeding orchards with the function of supplying modest numbers of pedigree seedlings. These pedigree seedlings would continually increase the cutting orchard clonal stock and consequently provide continuing diversity for the production forest.

If seed orchard managers are to implement a technique such as cutting hedges, a procedure for screening select genotypes for their

ability to root from vegetative material will be of the upmost importance. Such a screening procedure will prevent the construction of cutting hedges using clones that produce low percentages of rootable cuttings.

If rooting ability is sufficiently heritable, any superior genotype A that has been screened out on the basis of a low rooting percentage could be crossed with genotype B exhibiting a higher rooting percentage. Some of the progeny produced from this cross would hopefully combine genotype A's superior characteristics with genotype B's rooting ability, to produce easily rooted, superior types. Through vegetative propagation the outstanding performance of certain trees could consistently be repeated by rooted cuttings, whereas sibs of outstanding trees will not consistently represent the performance of the original outstanding genotype.

Objectives

This study was designed to examine natural variation in the rooting ability of shortleaf pine and the physical characteristics of such adventitious roots. Variation both among and within provenances were examined.

The objectives of this study were: (1) to examine natural variation in the rooting ability of shortleaf pine provenances west of the Mississippi, and (2) to estimate heritability for rooting ability and for some physical characteristics of the roots of the vegetative propagules.

CHAPTER II

LITERATURE REVIEW

Several investigators have studied natural variation in southern pines, but a limited amount of literature exists concerning variation in rooting ability of southern pines. There is a definite void in the literature concerning the physical characteristics of the roots of propagules of southern pines. Previous studies involving variation in rooting ability of southern pines have generally looked at rooting in terms of percent rooted and/or survival during a given trial. Although the availability of basic research data concerning variation in stem and root characteristics of vegetatively propagated commercial forest species is lacking, the current thrust of research in the area of vegetative propagation is striving to accommodate this need.

Recent studies of vegetative propagation of difficult-to-root species, such as loblolly pine, have shown that intensive testing and evaluation of those procedures and variables affecting propagation can often lead to successful rooting of cuttings.

Physiology of Rooting

Interest in the rooting ability of woody species has existed for many years, but this interest has become revitalized in recent years. Renewed interest is probably due to a growing recognition of the opportunities vegetative propagation offers to tree improvement research,

and the increased realization that rooting some of these species is becoming technically possible.

Researchers have found varying degrees of difficulty in rooting different species of pine; some root with relative ease from vegetative material while others seem to be impossible to root from a practical standpoint. There are a multitude of factors affecting the rooting of pine cuttings and many of these factors vary from one species to the next. Since the success of rooting is highly dependent upon the procedures used in collecting and handling the propagation material, a detailed look at current literature concerning each factor and its probable effects on rooting of pine is necessary.

Root development is a function of both internal and external factors (environmental conditions). It is the combination and interaction of these factors that determine the proficiency with which vegetative cuttings will root.

In vegetative propagation it is only necessary that a new root system be formed, since a potential shoot system is already present. Snyder (1974) listed three critical factors affecting root formation on vegetative cuttings. The cuttings must: (1) possess cells capable of dividing and differentiating into root initials. (2) possess favorable internal conditions, and (3) be placed in favorable environmental conditions.

Meristematic and parenchymatous cells are capable of differentiation. Parenchymatous cells, although not as differentiated or specialized as meristematic cells, may, under proper conditions, revert to meristematic cells (dedifferentiation). Vegetative propagation in woody species is possible because of the large abundance of

parenchymatous cells near the vascular tissue (Snyder, 1974). Cameron and Thomson (1969) found that prior to development of root initials, callus tissue develops from the vascular cambium, cortex, and pith at the base of the cutting in Pinus radiata D. Don. Callus is an irregular mass of parenchyma cells in various stages of lignification. In some hardwood and pine species callus tissue is not a precursor to rooting. Formation of callus tissue takes place at the same time as root initiation because both require similar environmental and physiological conditions (Hartman and Kester, 1975). However, in other species such as shortleaf pine, the formation of callus tissue is a precursor to root formation. After dedifferentiation (heterogenetic induction) of parenchyma cells near the vascular cambium, mitotic cell division begans the asexual reproduction process from which callus tissue is produced. Upon emergence from the callus tissue, the root cap and initial root tissues (root primordia) have already developed along with a complete vascular connection with the originating stem.

Internal factors affecting pine root growth include the availability of carbohydrates, nitrogen, auxin, and rooting co-factors (Snyder, 1974; Hess, 1965; Salisbury and Ross, 1978). Carbohydrates have two functions, energy storage and strengthening of cellular structure. There is considerable evidence that the carbohydrate level in a vegetative cutting exerts a strong influence on the development of the new root system (Hartman and Kester, 1975; Snyder, 1974). Rooting ability is maximized when the carbohydrate concentration in the parent tree is highest.

Nitrogen is necessary for nucleic acid and protein synthesis (Salisbury and Ross, 1978). Excessively high or low levels of nitrogen

were found to be detrimental to rooting (Hartman and Kester, 1975). Snyder (1974) reported that studies have shown that moderate levels of nitrogen in the stem have proven to be beneficial to rooting.

Indole-3-acetic acid (IAA), which is a naturally occuring auxin, was found to be one of several synergetic agents promoting root development on vegetative material (Thimann and Koepfli, 1935; Thimann and Went, 1934). Two synthetic auxins, indolebutyric acid (IBA) and naphthaleneacetic acid (NAA), developed around 1935, proved to be more effective than IAA in promoting rooting (Hartman and Kester, 1975).

Studies conducted by several researchers (Hess, 1965; Heuser and Hess, 1972; Gorter, 1969; Challenger, Lacey, and Howard, 1965) indicate that certain compounds (co-factors) found in the foliage and buds of juvenile plants, and compounds that appear in stem cuttings after treatment with IBA, have a synergistic effect with IAA in promoting rooting. Three of the compounds identified are oxygenated terpenoids, chlorogenic acid, and a phenolic compound catechol. The presence and abundance of these internal factors are dependent upon time of year, environmental conditions, and the plant's ability to synthesize then under given conditions.

The external factors which enhance rooting are favorable temperature, light, and moisture. Temperature affects photosynthesis which consequently affects the internal factors necessary for promoting rooting. The optimum temperature for rooting of vegetative material is 20 to 25 degrees Centigrade for most pine species (Albert, 1975).

Light is essential for the manufacture of chlorophyll, carbohydrates, and auxin (Speigel, 1955). Light intensity and duration must be sufficient to replenish carbohydrates used for respiration. Since

cuttings propagated in a greenhouse will recieve light of sufficient intensity for photosynthesis during normal daylight hours, supplemental light is more important in terms of the duration. Studies such as that by van Buijtenen et al. (1975) have shown that for loblolly pine, a long day photoperiod is the most effective for vegetative propagation. Waxman (1958) suggested that the light intensity be no less than 30 foot-candles for vegetative propagation of Cornus florida Rubra.

One of the greatest advancements in vegetative propagation came in 1940 with the development of the intermittent mist system (Gardner, 1941). Foliage of cuttings put under mist were found to be 10 to 15 degrees cooler than the foliage of cuttings not under mist (Langhans, 1955). Evaporation of the water on the needles absorbs heat from the needles which lowers the transpiration rate (Salisbury and Ross, 1978). If the temperature of the cutting can be lowered during photosynthesis, stored reserves will build up faster since the lower foliage temperature will reduce respiration which uses up those stored reserves. The intermittent mist also helps provide a very uniform air temperature across propagation beds.

There are many other factors directly related to the success of rooting cuttings. Ortet age is a very important factor influencing success in rooting pine cuttings. Depending on genotype and species, cuttings taken from pine trees less than nine years of age have a potential for 100 percent rooting, but as the age of the parent tree increases, rooting percentage declines (Black, 1972). Black further reported that although age was a limiting factor, genotype was the most important factor affecting the rooting ability of cuttings.

Yim (1962) suggested that the decrease in rooting ability as the

age of the parent tree increased might be explained by the content of indole-3-acetic acid (IAA) present. He observed that the highest content of IAA in the terminal buds of Pinus rigida Mill. was present in one year old seedlings and became progressively lower to age 17.

The best time of the year in which to collect pine cuttings for vegetative propagation is during the spring, as soon as the needles of the first flush have become fully extended and the shoots have attained some degree of maturity (Reines and Bamping, 1960).

Geographic and Clonal Variation Related to Rooting

The following studies report on geographic and clonal variation in shortleaf pine and other species. These studies were included to examine the current status and direction of research in the area of geographic variation which may be related to rooting ability and the propagules ability to survive after field planting.

Wells and Wakely (1970), using ten year plantation data representing 23 seed sources throughout the range of shortleaf pine, described well defined patterns of geographic variation in survival, total height, volume, and degree of early cone production. They found that differences in survival attributed to seed source were greatest in plantings in New Jersey, Pennsylvania, and Missouri where northernmost sources survived best and the southernmost sources survived the poorest.

Posey and McCullough (1969), reporting ten year results from the Southwide Pine Seed Source Study of shortleaf pine, observed an east-west trend in survival in an Oklahoma planting. They found a range in

survival from 67 percent for the most eastern source to 84 percent for the most western source. Another Oklahoma planting representing a north-south transect revealed a north to south trend with survival ranging from 86 to 96 percent, respectively. These trends suggest that possible geographic variation in the root structure of pine seedlings provide some indivivuals with a more optimum root system for the utilization of available moisture and nutrients.

The plant hormone auxin (IAA) has been found to stimulate root formation, therefore anything affecting the endogenous level of IAA in the stem could subsequently affect root formation. When IAA's function in the vegetative stage of plant growth is completed, the plant has two processes to remove the IAA concentration and limit buildup: (1) binding of IAA with other molecules to form derivatives and (2) degradation of IAA (Salisbury and Ross, 1975). In the degradation of IAA, IAA oxidase is the enzyme that catalyzes the process. Certain phenolic compounds called IAA oxidase inhibitors prevent the degradation of IAA and allow IAA to build up when needed to stimulate growth (Salisbury and Ross, 1978). Allen (1968) looked at racial variation in physiological characteristics of shortleaf pine roots of eight geographic seed sources. He reported significant racial variation in the content of pigment, IAA oxidase and peroxidase, IAA oxidase inhibitor, acetone powder, elongation inhibitor, and root respiration. Such variation in the biochemical constitution of the roots increases the possibility of geographic variation in rooting ability, although the degree to which this variation affects rooting ability has yet to be determined.

Variation in root structure of 13 varieties of Scotch pine (Pinus

sylvestris L.) represented by 45 provenances, grown in a greenhouse, was reported by Brown (1968). He concluded that some characteristics, such as location of lateral root development along the main tap root and root length, could be correlated with the climatic conditions of the provenance for northern European origins. The same root characteristics of the central European sources could not be correlated with climate. These differences varied greatly within individual sources and among different varieties.

Several studies have reported clonal variation in rooting ability. Farmer and Wilcox (1968) reported that for 49 clones of cottonwood (Populus deltoides Bartr.) tested in Mississippi, variation in early root development was under fairly strong genetic control and therefore responsive to selection. They reported that the high correlation between root characteristics, e.g. number of roots, total length of roots, and dry weight of roots, indicated that these measurements would be useful in evaluating rooting ability of the clones.

Zsuffa (1973), reported considerable variability in rooting ability of Pinus strobus L. and Pinus griffithii McClelland X strobus hybrids. In a series of trials, he found the same trees consistently rooted well or poorly, and he concluded that such rooting trials would be a good procedure for selection of easily-rootable types.

Sorensen and Campbell (1980) reported clonal variation in rooting success using cuttings from one year old western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings cultured in a glasshouse. Forty cuttings from each of three open pollinated families were placed in a rooting box using a randomized block design with five replications. They found no significant differences for provenance or family-in-

provenance, but siblings-in-family-in-provenance variance was highly significant. They reported that the results indicated that dominance and unique clone-effects were important to rooting success and that additive genetic effects were not. This indicates that there is a need for closer evaluation of individual clones in terms of their rootability if vegetative propagation is to become a tool for tree improvement.

In summary, research results to date indicate the need for an explicit understanding of the physiological processes of vegetative growth and the procedures used to successfully root vegetative material. Studies of shortleaf pine concerning traits such as total height, volume and degree of early cone production demonstrate that racial variation does exist for many characteristics of the species. Several authors have reported unique clonal effects on rooting of vegetative material. If there is considerable variability in rooting ability from tree to tree and if rooting ability is sufficiently heritable, then individual tree selection for rooting ability should provide a way to increase productivity when using vegetative propagation. Those studies which found provenance differences in survival and physiological characteristics of the roots suggest the possibility of racial variation in root structure and rooting ability of shortleaf pine. If geographic and/or clonal variation in rooting ability and root structure exist, there is a need for evaluation of these characteristics to determine if selection would be practical for tree improvement purposes.

CHAPTER III

MATERIALS AND METHODS

Cuttings for the rooting trial were collected from 22 geographic locations (provenances) across the five western states of shortleaf pine's natural range (Figure 2). The points of collection were selected using a grid composed of mean annual precipitation isolines and degrees in latitude. At each location ten cuttings were collected from each of 13 ortets, making a total of 130 observations per provenance.

The number of cuttings needed to reliably estimate heritability was determined by using the following formula derived by McNew (1980) from the Department of Statistics at Oklahoma State University.

$$n = \frac{(T+1) + (T-1)\ h^2}{2 + (T-1)\ h^2}$$

Where

n = Number of cuttings per tree needed to estimate heritability (h^2) with T number of total observations. The estimate used for h^2 was an average of h^2 available for all characteristics of interests, since only one n can be utilized. The estimated average of h^2 used was 0.12.

T = 2,860 which was the total number of cuttings which could be handled efficiently under our given space and time constraints.

n was estimated:

$$n = \frac{(2{,}860+1) + (2{,}860-1)\ 0.12}{2 + (2{,}860-1)\ 0.12} = 9.29$$

or n = 10 cuttings per tree

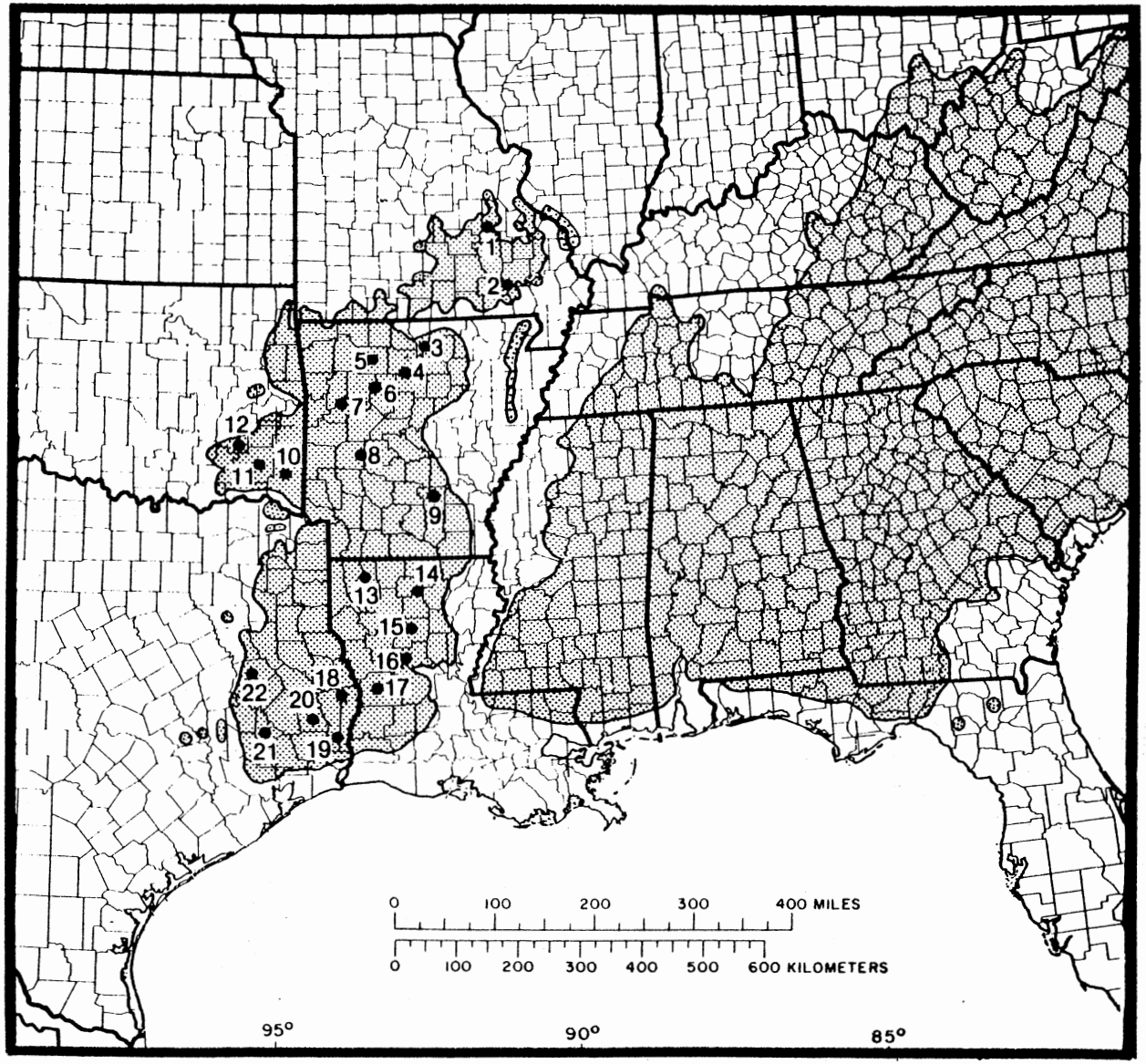


Figure 2. Geographic location of collection points and western range of shortleaf pine.

When the physiological state of the current years growth had reached the point of maturity desired (June 2, 1980), two crews began the process of collecting the cuttings. Crew one was to begin collection in Oklahoma, then head east through Arkansas and north to Missouri. Initially crew one was to collect all the cutting locations in these three states (locations 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12) except for the southernmost location in Arkansas. Crew two was to collect from the locations in Texas, Louisiana, and the southernmost location in Arkansas (locations 9, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22). Each crew would have collected from 11 locations, within a five day period, exact timing depending upon the distance between locations and the time required to find a cutting site at each location.

Due to uncontrollable logistic problems, crew one was unable to collect from the collection points in Missouri and Oklahoma (locations 1, 2, 10, 11, 12). After crew two had collected its original points and returned to the study location, they took crew one's collection box and collected the Oklahoma and Missouri collection points. This added an additional four days to the collection procedures and consequently added one extra day to the potting process since work schedules had to be rearranged for those persons helping pot the cuttings. All cuttings remained in the collection boxes under the same conditions until collection was over. The only change due to the logistic problems was the time difference between collection and potting which posed no serious problem since the time factor could be delt with in the analysis.

The cutting requirements for collection were; minimum cutting length of six inches, maximum age of ortet of eight years, minimum

number of ten cuttings per tree, and no visible disease or insect damage. Cuttings from each tree were labeled by cutting location and ortet number, then packed in transportation boxes.

The transportation boxes consisted of two stripped-down refrigerator bodies, which when placed horizontally provided a large, well insulated chest. The inside of the refrigerators were fitted with a wooden perimeter frame to support metal trays which held ice bags four to six inches above the cuttings during transportation. Six holes were drilled into each box to provide good drainage and then a six inch layer of perlite and vermiculite (50/50 mix) was added. This six inch layer provided a medium capable of holding moisture and a medium in which the cuttings could be kept in a vertical position. Grigsby (1971) reported that by maintaining an upright position throughout all handling, rooting success was improved. The ice kept the air temperature around the cuttings near 40 degrees Fahrenheit, and the melting provided adequate moisture for the cuttings.

Four hundred sixty cuttings were needed to provide a two row border, so two extra cuttings per tree were collected to provide a total number of 3,320 cuttings.

Mist Chamber Design

The experimental design and the number of cuttings involved required a large rectangular table (propagation table) that would hold the 140 flats needed for this experiment. Each flat was a plastic tray containing 25 2.5 square-inch plastic pots that were four inches deep. A rectangular table 22.75 x 14.25 feet was built. To build this table (propagation table), eleven 6 x 4.75 foot redwood frames

with expanded metal tops were arranged in a rectangular design with concrete blocks supporting each frame as shown in Figure 3. The sides and ends of this rectangular table had four inch galvanized flashing attached to provide a three inch high barrier encircling it. The top of the propagation table was then covered with six mill polyethylene perforated for drainage. Heating cables were laid three inches apart on the bed of the table, running the full length of the table, to provide bottom heat for the cuttings. When this was completed, a 2.5 inch layer of perlite and vermiculite in a 50/50 mix was poured on the table. Figure 4 illustrates the propagation bed design. This layer of perlite and vermiculite on the bed of the table served two purposes; first to cover the heating cables so that the flats would not set directly on top of them and second, to provide a medium with water holding capacity so that the humidity directly around the cuttings could be maximized.

To prepare an optimum environment for the cuttings, an enclosure was built that would make it possible to control humidity and light, and to provide a structure from which a mist system could be supported. The mistroom structure is illustrated in Figure 5.

Five redwood 2 x 4's acting as stability beams (connecting the two longest sides of the mistroom at the top of the walls) provided structures for the lights and the misting apparatus to be attached. Figure 6 illustrates the mistroom interior. Wood blocks one foot high were centered at the top of each end wall and a beam running the full length of the mistroom was attached to these blocks. When polyethylene was stretched across the top, this center beam provided a sloping roof so that condensation on the ceiling would run off to the sides, pre-

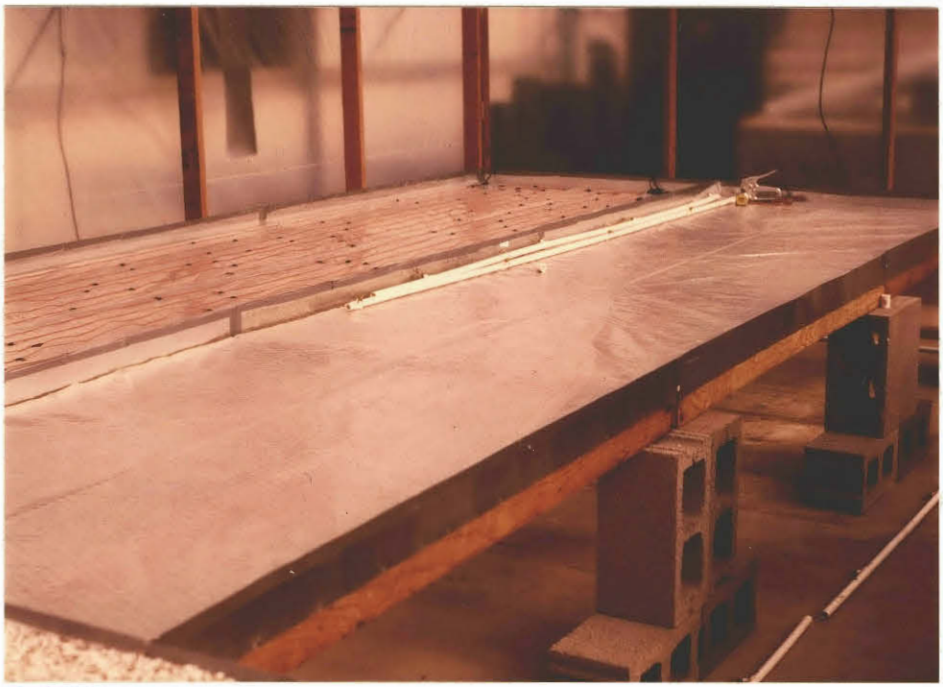


Figure 3. Propagation Table Design

Figure 4. Propagation Bed Design

Figure 5. Mistroom Structure

Figure 6. Mistroom Interior

venting dripping and subsequent washing of portions of the table.

Inside the chamber ten florescent lights (two 40 watt bulbs/light) were used to extend the daylength period to 16 hours. This was the suggested daylength period for the production of loblolly pine from vegetative material (van Buijtenen et al., 1975). The lights were spaced 25 inches apart at 40 inch intervals, and 32 inches above the propagation bed. The lights were activated at 5:00 a.m. and shut off at 9:00 p.m. with a time clock. A minimum of 50 foot-candles was provided during the dark hours of the artificial photoperiod.

Cooling was provided by a 12 inch fan located in the center of one of the side walls and an air inlet was located in the center of the opposite wall. Cool air was provided by the greenhouse cooling system.

The mist system consisted of 24 0.020 x 1/8 inch foggers. The nozzles were mounted on three lines of PVC (schedule 40) pipe using PVC saddles. The lines were spaced 47 inches apart, with the nozzles spaced at 32 inch intervals. The lines were suspended 20 inches above the cuttings. Since the nozzles were pointed up, the tops of the nozzles were approximately 23 inches above the cuttings. The type of coverage provided by this mist nozzle configuration is shown in Figure 6. The nozzles were activated two ways: (1) When the thermostat turned on the chamber fan, it also activated the time clock that controlled the nozzles. This time clock activated the nozzles six seconds out of every six minutes. (2) Since the fan would not operate on cool days a separate time clock activated the spray periodically (every two hours). These conditions provided an average relative humidity of 90 percent and an average daily temperature of 75 degrees Fahrenheit

directly around the cuttings. The heating cables provided a bottom heat of approximately 79 degrees Fahrenheit.

Potting Procedures

Each flat containing 25 pots was filled with a 50/50 mix of perlite and vermiculite and then placed on the table so that rows and columns of pots could be easily distinguished. A completely randomized planting design was chosen for this study as the most appropriate design for analysis of data with missing values. The perlite-vermiculite mixture provided a medium with the oxygen and water retention capability desirable for rooting cuttings. The prefilled planting pots were placed in the chamber prior to collection of the cuttings so that the mist system could saturate the potting medium and the mistroom conditions would stabilize before the cuttings were potted.

A six inch plastic pot label was used to identify each individual cutting in the chamber. Randomization of the cuttings was accomplished by placing all premarked labels in a large container and shaking it well to mix the labels. Each label was then randomly drawn from the container and matched with a cutting from the collection box with a corresponding label. Prior to potting each cutting had its basal inch of needles stripped off, was measured for total length, weighed to the nearest tenth gram, checked for any growth from the previous year, dipped in a rooting hormone (Hormodin 3, 0.8 ppm IBA), and then potted with its corresponding label. Captan powder (50 percent active ingredient by weight) was added to the rooting hormone to prevent rotting of the base of the cuttings. Each label described the cutting location, ortet number, and cutting number. In addition to the label, each

cutting was assigned a row and column on the table. The purpose of this procedure was to (1) provide a logical sequence for recording data and (2) help identify any patterns of survival and/or mortality that might originate due to micro-environmental differences within the chamber.

Treatment of Cuttings

After the cuttings were planted, a modified Hoagland's solution (Hoagland and Snyder, 1934) was used as a nutrient additive and applied as a foliar drench once a day by hand spraying. The composition of the nutrient solution was the same as used by van Buijtenen et al. (1975) and is given in Table I.

TABLE I

COMPOSITION OF NUTRIENT SOLUTION

Element	ppm
N	50
K	50
P	150
Ca	50
Mg	20
Cu	0.016
B	0.01
Mn	0.025
Zn	0.1
Mo	0.01
Fe	5.5

During the first week of the study, a test of the pH of the water supply at the greenhouse revealed a higher than desirable pH level (8.9). Immediate steps were undertaken to lower the pH level of the water supply. A Merit Commandor proportioner pump was installed to induce a 1/100th molar sulfuric acid solution into the mist lines. Due to the hammer effect produced by the water pressure coming on and off during the misting of the cuttings, the Commandor pump began to lock up and failed to induce the acid solution into the mist line two weeks after installation. The only option then was to hand spray the acid solution twice a day to offset the pH balance as much as possible.

Due to the high humidity and the presence of a nutrient supply, a minor fungal problem developed during the third and fourth week of the experiment. To eliminate the fungus and prevent spreading of the spores, a dilute solution of Benlate was sprayed twice during those two weeks. No apparent damage due to the fungus was observed on the cuttings.

Measurements

Measurements and information recorded prior to planting were:

(1) INITIAL CUTTING LENGTH (measured to nearest 0.1 centimeter)

(2) INITIAL CUTTING GREEN WEIGHT (measured to nearest 0.1 gram)

(3) PRESENCE OF LAST YEARS GROWTH (yes or no)

(4) TIME FACTOR (number of days between collection and first day of planting)

The cuttings remained in the propagation bed for 106 days (June 14, 1980 to September 29,1980). After the cuttings had been in the mistroom for 100 days, four days of spot checking the border row

cuttings indicated that the cuttings had been in the mistroom long enough to allow sufficient root development for measurement of the characteristics of interest. Van Buijtenen et al. (1975) reported that rooting percentages increased up to 14 weeks (98 days) then tapered off at an increasing rate.

After 106 days the propagules were lifted in the same order as they had been potted so as to equalize, as best as possible, the exact number of days each cutting remained in the mistroom. This procedure was appropriate since it took five days to pot the cuttings and four days to remove them. Each individual cutting was carefully removed from its container and if a root structure was present, a slow agitation of the rooted cutting in water easily removed any perlite and vermiculite that was packed around the roots. As each cutting was lifted it was classified according to its physiological state: (1) dead, (2) alive, (3) callused, or (4) rooted. The basis of classification was as follows:

(1) Dead: cuttings not rooted; with 2/3 of the cutting's needles non-turgid, brown; with signs of rotting along the stem.

(2) Alive: cuttings not rooted; with 2/3 of the cutting's needles turgid, green; with no signs of rotting along the stem.

(3) Callused: cuttings in the same physical condition as those classified alive; in addition, visible formation of callus tissue at the base of the cuttings.

(4) Rooted: those cuttings exhibiting a root structure.

Data recorded immediately after lifting were:

(1) TOTAL NUMBER OF ROOTS (roots 0.5 centimeters or longer were tallied as one root each)

(2) TAP ROOT LENGTH (measured to the nearest 0.1 centimeter)

(3) TOTAL ROOT LENGTH (measured to the nearest 0.1 centimeter including tap root length)

(4) ROOTED CUTTING STEM DIAMETER (measured to the nearest 0.001 inch)

After completion of the above measurements, the roots from each cutting were placed in a plastic bag and then in a paper sack along with the stem and the corresponding label. The sacks containing the study material were transported to the lab for determination of stem and root dry weights.

Measurements taken in the laboratory were:

(1) ROOT DRY WEIGHT (measured to nearest 0.001 gram)

(2) ROOTED CUTTING STEM DRY WEIGHT (measured to nearest 0.001 gram)

Preliminary drying trials showed the stems reached a constant dry weight at an oven setting of 25 degrees centigrade after 32 hours. Since the roots had considerably less mass than the stems, they reached a constant dry weight at an oven setting of 25 degrees centigrade after 26 hours. As each batch of stems and roots were removed from the ovens, they were placed in desicant jars to prevent moisture gain prior to weighing.

Statistical Analysis

Data were compiled and sorted by source, tree, and cutting, using the Statistical Analysis System (SAS) programming language (Barr and

Goodnight, 1979). Procedure Nested of the (SAS) system computes analyses of variance and covariance for experiments with a nested (hierarchical) structure and was used in the analysis of this study. Procedure Nested was selected for its efficiency in analyzing designs with large numbers of levels and observations. The Nested procedure provided means, estimates of variance components, and estimates of variance component correlations.

All root and stem characteristics were tested for significance at the .05 level of probability. Correlations between all root and stem characteristics listed were significant at the .05 level of probability. Table II illustrates the analysis of variance and procedure for estimating heritability.

TABLE II

PROCEDURE FOR ANALYSIS OF VARIANCE
AND ESTIMATING HERITABILITY

Source of Variation	d.f.	Expected Mean Squares*
Source	$s-1$	$\hat{\sigma}^2_{c/o/s} + K_2\hat{\sigma}^2_{o/s} + K_3\hat{\sigma}^2_{s}$
Ortet in source	$\sum_{i=1}^{s} (o_i-1)$	$\hat{\sigma}^2_{c/o/s} + K_1\hat{\sigma}^2_{o/s}$
Cutting in ortet in source	$\sum_{i=1}^{s} \sum^{i}_{j=1} (n_{ij} -1)$	$\hat{\sigma}^2_{c/o/s}$

$$h^2_{bs} = \frac{\hat{\sigma}^2_{o/s}}{\hat{\sigma}^2_{c/o/s} + \hat{\sigma}^2_{o/s}}$$

*K_1, K_2, K_3 are respective coefficients of the expected mean squares.

s = source, o = ortet, c = cutting

CHAPTER IV

RESULTS AND DISCUSSION

This study was designed to examine both the geographic and tree to tree variation in rooting ability and physical characteristics of the roots of vegetative propagules of shortleaf pine. A total of 2,860 shortleaf pine cuttings were collected and placed in a controlled environment. Of those 2,860 cuttings, 586 or 20.5 percent rooted, providing information on all 22 sources.

Rooting Ability

The percent of ortets producing at least one rooted cutting was 52.1 or 149 of the 286 ortets tested. Tables III and IV present the percent of cuttings, by source, that produced at least one root, and the percent of ortets, by source, that produced at least one rooted cutting, respectively. The characteristics of interest were the percent of cuttings rooted and percent of ortets rooted. The analysis of variance for these characteristics were based on the all-or-none form of the data.

The analysis of variance for percent of rooted cuttings and percent of ortets rooting showed no significant source differences. The percent of rooted cuttings did show significant ortet in source differences, indicating clonal differences in rooting ability. These data suggests that improvement in rooting ability is possible through

TABLE III

PERCENT OF CUTTINGS (BY SOURCE) THAT PRODUCED AT LEAST ONE ROOT

Missouri = MO
Arkansas = AR
Oklahoma = OK
Louisiana = LA
Texas = TX

MO Source 1 = 20.76	OK Source 12 = 12.30
MO Source 2 = 16.15	LA Source 13 = 16.92
AR Source 3 = 13.84	LA Source 14 = 20.76
AR Source 4 = 16.92	LA Source 15 = 33.07
AR Source 5 = 17.69	LA Source 16 = 26.15
AR Source 6 = 13.84	LA Source 17 = 31.53
AR Source 7 = 18.46	TX Source 18 = 23.07
AR Source 8 = 29.23	TX Source 19 = 16.15
AR Source 9 = 28.46	TX Source 20 = 20.00
OK Source 10 = 11.53	TX Source 21 = 23.07
OK Source 11 = 17.69	TX Source 22 = 23.07

Average percent of rooted cuttings = 20.49

Average standard error for source percentages = $\pm$3.48%

TABLE IV

PERCENT OF ORTETS (BY SOURCE) PRODUCING
AT LEAST ONE ROOTED CUTTING

Missouri = MO
Arkansas = AR
Oklahoma = OK
Louisiana = LA
Texas = TX

MO Source 1 = 53.85	OK Source 12 = 23.08
MO Source 2 = 46.15	LA Source 13 = 53.85
AR Source 3 = 53.85	LA Source 14 = 46.15
AR Source 4 = 46.15	LA Source 15 = 92.31
AR Source 5 = 53.85	LA Source 16 = 61.54
AR Source 6 = 30.77	LA Source 17 = 69.23
AR Source 7 = 38.46	TX Source 18 = 53.85
AR Source 8 = 69.23	TX Source 19 = 38.46
AR Source 9 = 61.54	TX Source 20 = 53.85
OK Source 10 = 30.77	TX Source 21 = 69.23
OK Source 11 = 38.46	TX Source 22 = 61.54

Average percent of rooted ortets = 52.09

Average standard error for source percentages = $\pm$13.11%

individual tree selection, but that the testing and selection of different origins is apparently not important.

For comparison purposes, percent of cuttings producing at least one root were averaged by state. The order in which the states ranked, based on their average percent of rooted cuttings, was Oklahoma 14, Missouri 18.5, Arkansas 19.7, Texas 21, and Louisiana 25.8 percent. The order in which the states ranked based on their average percent of ortets producing at least one rooted cutting were Oklahoma 30.7, Arkansas 49.4, Missouri 50, Texas 55.4, and Louisiana 64.6 percent. A slight trend from north to south seems evident when looking at these values for rooting; however, source differences were not significant. Analysis of rooting ability by state was evaluated to determine if these apparent differences in rooting ability by state were statistically significant. The results of this analysis indicated that these differences were not significant. One possible reason for the apparent trend in rooting ability, even though source and state differences were not significant, might be the following. When examining rooting ability, it is evident that there is a large amount of variability in rooting ability among ortets within sources. As stated earlier, ortet in source differences were significant. When using the nested procedure of analysis, the proper error term for testing source differences is among ortets in source. The possibility exists, since the error term (ortets in source) was large, that the analysis was unable to detect significance for this apparent trend. In other words, the variance component attributed to ortets in source was very large compared to the variance component attributed to source. The important point, however, is that clonal variability in rooting ability is

considerable, while source variability is small in comparison.

Figure 7 graphically presents the percent and number of cuttings in each of the four physiological categories as assigned at the termination of the study. Under optimum rooting conditions, the large number of callused (22 percent) and alive cuttings (14 percent) would not be expected and a larger number of rooted cuttings might be expected. The expectancy of a higher percentage of rooting was based on results of studies that have reported up to 60 percent rooting for loblolly and shortleaf pine (van Buijtenen et al., 1975; Greenwood et al., 1980). The high percentage of cuttings callused and alive but not rooted, indicate that a suboptimal level of some factor or factors affecting rooting was present during the study. Two factors considered to have been present in suboptimal form were the pH of the water and amount of water applied. Hartman and Kester (1975) reported that a high pH tends to adversely affect callus tissue and consequently reduce rooting. The amount of mist applied was determined to be the most detrimental based on an article published by Greenwood et al. (1980) at the conclusion of this study. Greenwood et al. (1980) reported that the optimum mist regime for loblolly and shortleaf pine cuttings was found to be .05 to .10 millimeters of water per hour. They further reported that the amount of mist applied was the most critical factor of the environment effecting rooting. The amount of mist applied in this author's study was determined to be 2.7 millimeters per hour maximum; however, the mist system used was operated at the lowest calibration possible with the time clock available. A study on the propagation of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) cuttings in a variety of media (Copes, 1977) also indicated

(Percentages based on a total of 2,860 cuttings)

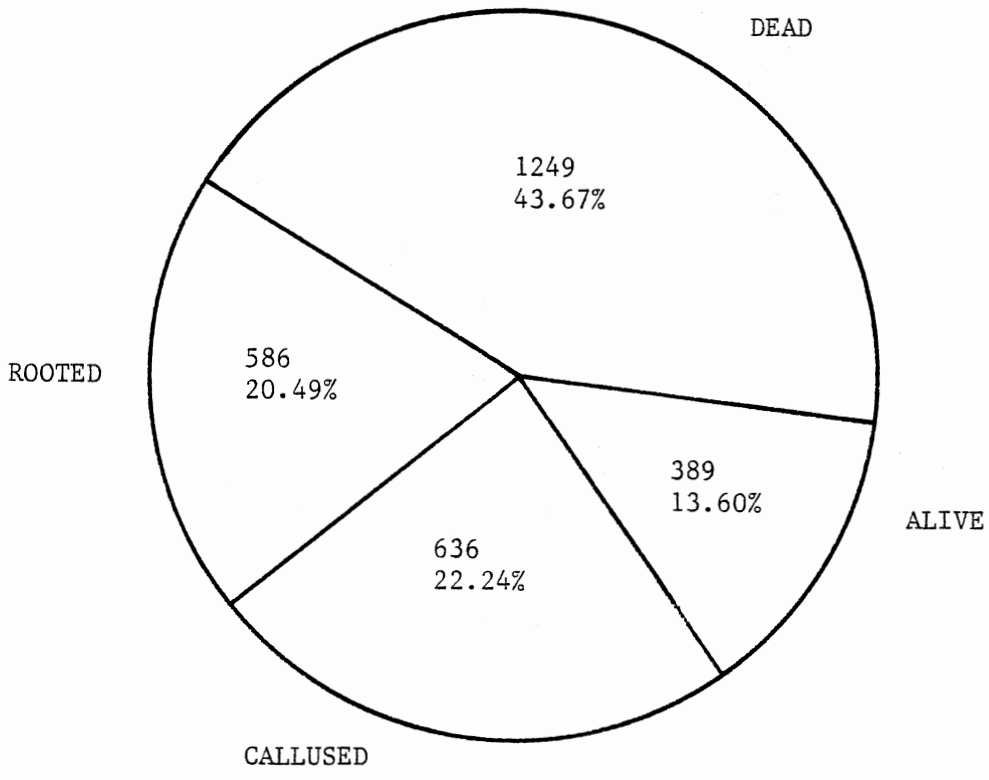


Figure 7. Physiological State of Cuttings at Conclusion of Study

adverse affects of too much water on vegetative cuttings. Copes (1977) reported lower rooting percentages from media that retained too much or too little water. Based on these results, it is evident that a suboptimal rooting environment was present, both in quantity of water applied and in pH. An excessive amount of mist probably resulted in an adverse affect on the concentration of plant growth regulators within the cuttings.

Because of the logical assumption that under optimum rooting conditions some of those cuttings callused, and possibly some of those alive, would have rooted, the following analyses are presented.

The analysis of the cuttings callused showed that source differences were not significant and that ortet in source differences were significant. If one also assumes that a percentage of the cuttings alive would have formed callus tissue and proceeded to root, examination of this category becomes of interest. In the analysis of the cuttings classified alive, source differences were significant and ortets in source were not.

At this point it was necessary to determine if the results of the rooted cuttings analysis would have been changed if those cuttings classified alive and callused had rooted. To accomplish this, two separate analyses of rooting ability were run in which the cuttings classified as callused and rooted, and alive, callused, and rooted were combined and evaluated as two separate groups. The results of both of the combined analyses were the same as the results of the analysis for the rooted cuttings only. Source differences were not significant and ortet in source differences were significant. These results give support to the earlier conclusion that clonal differences

in rooting ability are important and that source differences are not.

Root Characteristics

Four root characteristics of the rooted propagules were measured to examine the geographic and/or clonal variation in those characteristics. The four root characteristics measured were total root number, tap root length, total root length, and root dry weight. In addition to the four root measurements taken, five stem measurements were taken; initial cutting length, initial cutting green weight, presence of old growth, rooted cutting dry weight, and rooted cutting diameter. The five stem characteristics measured were of interest in terms of their relation to rooting ability and the root characteristics of the rooted cuttings. These characters are evaluated and discussed in the section on correlations between root and stem characteristics. Table V presents means for all root and stem characteristics by source and averaged over sources.

The four root characteristics showed no significant source differences. All four root characteristics did show significant variation among ortets in source. Table VI presents the observed mean squares and F test results for all root and stem characteristics from the analyses of variance. These data suggest that the root system or structure of pine propagules can be modified through clonal selection of any of the four root characteristics measured. When clones producing root characteristics that are favorable to establishment and survival are identified, (for a range of environments or a specific environment depending upon a researcher's goals), those clones can be selected and used to develop clonal stock for specified sites.

TABLE V

MEANS FOR ALL ROOT AND STEM CHARACTERISTICS BY SOURCE AND AVERAGED OVER SOURCES

SOURCE	INITIAL CUTTING LENGTH	INITIAL CUTTING GREEN WEIGHT	ROOTED CUTTING STEM DIAMETER	ROOTED CUTTING DRY WEIGHT	TAP ROOT LENGTH	TOTAL ROOT LENGTH	TOTAL ROOT NUMBER	ROOT DRY WEIGHT	CUTTING UNIT WEIGHT†	ROOT UNIT WEIGHT††
1	18.20	9.748	0.1815	1.8677	5.7777	22.129	7.2222	0.0420	0.5365	0.002365
2	16.25	9.399	0.1907	1.5523	5.3904	13.052	5.0000	0.0333	0.5813	0.002796
3	16.25	8.096	0.1503	1.1103	4.7222	7.088	2.6666	0.0416	0.4998	0.005381
4	17.75	8.780	0.1884	1.5360	4.6090	11.368	5.8181	0.0351	0.4983	0.003386
5	15.89	6.924	0.1572	1.1431	6.8304	23.834	7.9130	0.0496	0.4381	0.002244
6	18.73	7.863	0.1652	1.6677	4.4777	18.127	6.3888	0.0534	0.4256	0.002314
7	18.08	8.659	0.1805	1.7149	3.7375	10.187	3.7916	0.0476	0.4804	0.006256
8	17.18	7.245	0.1600	0.3457	6.0894	19.242	6.4473	0.0457	0.4226	0.003158
9	18.68	8.710	0.1813	1.6063	5.0216	19.808	7.1621	0.0426	0.4663	0.002816
10	17.00	8.630	0.1828	1.4950	4.0533	8.013	3.9333	0.0329	0.5134	0.003820
11	18.75	8.865	0.1391	1.3491	6.9565	15.130	4.0434	0.0617	0.4732	0.005221
12	14.34	7.629	0.1826	1.4922	5.6250	11.525	5.3125	0.0404	0.5389	0.003971
13	18.88	8.270	0.1682	1.7019	6.2090	17.263	7.5000	0.0425	0.4376	0.002436
14	19.94	7.796	0.1603	1.5054	5.5111	13.007	5.3333	0.0335	0.3992	0.002859
15	20.62	7.954	0.1704	1.5205	6.6255	28.200	11.8372	0.0655	0.3879	0.002612
16	20.87	8.310	0.1810	2.2579	5.6558	15.244	5.6470	0.0503	0.3935	0.003635
17	21.61	9.390	0.1773	1.8645	5.4463	21.007	7.9024	0.0606	0.4344	0.003764
18	20.82	8.695	0.1969	1.8937	5.4200	35.326	11.3666	0.0871	0.4224	0.002836
19	19.38	8.883	0.1791	1.7517	4.4095	7.228	2.0476	0.0199	0.4606	0.003333
20	19.90	8.596	0.1686	1.6068	4.5538	7.876	2.8076	0.0267	0.4560	0.003742
21	22.68	10.880	0.1952	2.1810	5.4066	12.020	3.6666	0.0370	0.4842	0.003837
22	22.31	9.433	0.1882	2.0777	4.4866	8.6466	3.3000	0.0338	0.4198	0.004790
AVERAGE	18.82	8.580	0.1752	1.6758	5.4049	16.803	6.1621	0.0464	0.4623	0.003472
UNITS	cm	gr	in	gr	cm	cm	no	gr	gr/cm	gr/cm

†CUTTING UNIT WEIGHT represents initial cutting green weight over initial cutting length

††ROOT UNIT WEIGHT represents root dry weight over total root length

TABLE VI

OBSERVED MEAN SQUARES AND F VALUES FOR ALL ROOT AND STEM CHARACTERISTICS

SOURCE	d.f.	TOTAL ROOT LENGTH	F	TOTAL ROOT NUMBER	F	ROOT DRY WEIGHT	F
TOTAL	585	469.42		66.82		0.00248	
SOURCE	21	1505.67	1.39	199.34		0.00615	1.16
ORTETS/S	127	1076.16	4.45*	144.07	3.79*	0.00528	3.54*
C/O/S	437	241.95		37.99		0.00149	

SOURCE	d.f.	TAP ROOT LENGTH	F	INITIAL CUTTING LENGTH	F	INITIAL CUTTING GREEN WEIGHT	F
TOTAL	585	10.3513		17.338		9.554	
SOURCE	21	19.4079	0.82	136.734	5.99*	30.346	2.58*
ORTETS/S	127	23.7127	3.93*	22.791	2.28*	11.741	1.48
C/O/S	437	6.0330		10.016		7.919	

SOURCE	d.f.	ROOTED CUTTING STEM DIAMETER	F	ROOTED CUTTING DRY WEIGHT	F	CUTTING UNIT WEIGHT	F
TOTAL	585	0.00201		0.42884		0.02390	
SOURCE	21	0.00547	1.14	2.42194	3.18	0.06924	2.72*
ORTETS/S	127	0.00387	2.98*	0.76092	3.22*	0.02549	1.20*
C/O/S	437	0.00130		0.23656		0.02125	

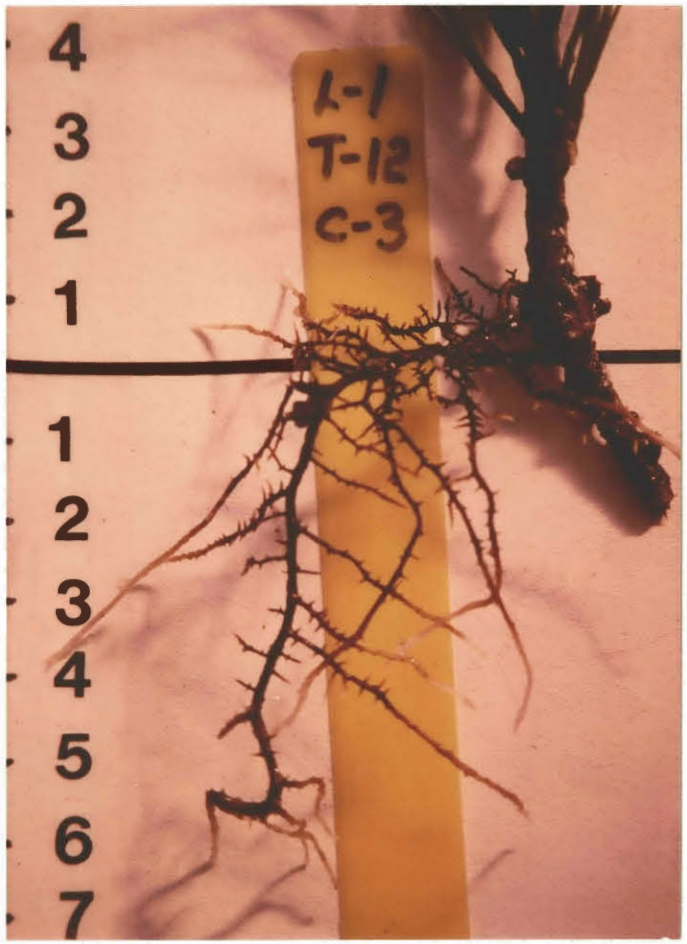
SOURCE	d.f.	ROOT UNIT WEIGHT	F	d.f.	PERCENT ROOTING BY SOURCE	
TOTAL	585	0.561456×10^{-5}		2859	0.16297	
SOURCE	21	0.280763×10^{-4}	3.07*	21	0.48978	0.90
ORTETS/S	127	0.913816×10^{-5}	2.60*	264	0.54033	4.44*
C/O/S	437	0.351115×10^{-5}		2574	0.12160	

*represents significance at the .05 level of probability or lower
S = Source O = Ortet C = Cutting

A root mass per unit root length ratio was evaluated using the total root weight and total root length characteristics. The root mass per unit root length ratio was found to be significantly different among sources. Since the ratio could have been interpreted as reflecting the different stages of development of the root systems present, an analysis using simple linear regression was applied using the variables root dry weight and total root length to determine regression lines for each of the 22 sources. Plotting the slopes of the 22 regression lines and subsequent testing showed significant differences among the slopes, providing evidence that the mass per unit length ratio of the roots was actually evaluating the presence of different structural characteristics of the roots from one source to another. Some of the root systems formed tended to have small fibrous roots while others exhibited large diameter, non-fibrous roots. Although neither of the individual characteristics of the ratio were found to be significant by source, the ratio should be interpreted (as indicated by the analysis) as a separate characteristic. The mass per unit length ratio of the roots was also statistically significant among ortets in source.

Given that these structural differences in root formation are maintained by the rooted propagules after field planting, both geographic and/or clonal selection for this characteristic could be useful when making selections for tree improvement programs. Since environmental conditions in the field are seldom optimum for establishment, clones that produce root systems more efficient in water and nutrient uptake could be selected to increase survival rates. Figure 8 illustrates the two most prominent types of root structures observed in

(a) Prominent Root Type I - Large Diameter, Non-Fibrous Roots Exhibited by Tree 11 from Arkansas (Source 9)



(b) Prominent Root Type II - Fibrous Type Root System Exhibited by Tree 12 from Missouri (Source 1)

Figure 8. Illustration of Two of the Most Prominent Types of Root Structure Observed

this study. Although source differences in root structure need to be further researched to determine its usefulness and practicality in a selection program, it could possibly make evaluation of origin important. There was no apparent geographic trend in the root mass per unit length ratio.

Correlations Between Root and Stem Characteristics

Estimates of the components of variance and variance component correlations were obtained from the mean squares and mean cross products of the analysis of variance and covariance. In addition to the four root measurements taken, five stem measurements were also taken to provide correlations between the different root and stem characteristics

Estimates of genetic correlations are of interest to the tree breeder because they indicate how selection for one character may affect or alter another character. For example, if a hard to evaluate root characteristic such as root number is highly correlated with an easy to measure stem characteristic such as cutting length, then a predetermined criterion for cutting length would subsequently increase root number. Only the correlations found to be significant at the .05 level of probability or lower are discussed in this section. All correlations discussed except those including the variables percent rooted or presence of old growth are genetic correlations.

There was a low negative correlation (-0.17) between the root mass per unit root length ratio and total root length. The correlation (even though small) suggests that as the mass per unit length ratio of the roots increases, there is a small decrease in total root

length. This supports the finding of differences in structural characteristics of the roots, as does the correlation between the mass per unit length ratio of the roots and root number. This correlation was also small and negative (-0.17), and suggests that as the mass per unit length of the roots increases, there is a tendency toward a decrease in the number of roots.

When the presence of old growth was correlated with the four root characteristics measured, all correlations were found to be nonsignificant. Old growth was also uncorrelated with rooting ability, indicating that the inclusion of old growth as part of the cutting material was not beneficial for this study.

Both initial cutting green weight and rooted cutting dry weight were uncorrelated with all four root characteristics measured. Initial cutting green weight was also uncorrelated with rooting ability. Since cutting dry weight could be measured on the rooted cuttings only, the correlation of cutting dry weight with rooting ability would be inappropriate.

Initial cutting length had a low negative correlation (-0.15) with tap root length. Since most research with vegetative propagation utilizes a standardized cutting length that ranges from one to ten centimeters shorter than the cuttings used in this study, and since the correlation was low, the effects of adjusting cutting length to favor tap root length would be minimal at best. Initial cutting length was positively correlated with percent of rooted cuttings (0.59). Since cuttings from the southern origins tended to be longer when collected, this correlation may be the cause of the apparent geographic trend in rooting ability. The correlation also suggests that selection for

longer cuttings, when collecting, could increase rooting success.

Because stem diameter was taken only on the rooted cuttings, the low positive correlations (0.18, 0.13, 0.12) of stem diameter with total root number, root dry weight, and total root length, respectively, might reflect vegetative growth of the stem following rooting. However, if this should be the case it is of interest that rooted cutting dry weight was uncorrelated with those same root characteristics. It is also possible that the formation of callus tissue caused a detectable swelling at the base of the cuttings, in which case a correlation of rooted cutting dry weight with those same root characteristics would not be expected. Since an initial stem diameter was not taken on all the cuttings, determination of the exact affect of stem diameter on these root characteristics cannot be made.

Seventy three percent of the cuttings that rooted had an initial cutting unit weight (cutting mass per unit cutting length) value of .22 to .69 grams per centimeter. For mass production purposes, the use of cuttings that fall into a similar weight to length ratio could be one method to increase rooting percentages.

When the percent of rooted cuttings was correlated with the time factor (the number of days between collection and planting), an unexpected, high positive correlation (0.52) was observed. This correlation suggests that as the number of days the cuttings were stored increased, rooting percentages increased. This correlation may also reflect varying stages of maturity of the cuttings collected. With the exception of Oklahoma sources 10 and 12 which had the shortest time factor value of one day, the next shortest time factor values (three days to five days) were represented by Missouri sources one and

two and Arkansas sources three, four, and five, which were the northernmost sources collected. Sources one through five would undoubtedly be composed of the least mature cutting material if a maturity difference was present. If a maturity difference was present and was the cause of reduced rooting in the least mature sources, then the observed correlation would result. Visual observations made of Oklahoma sources 10 and 12 during collection indicated that considerable tip moth damage the previous year had tended to retard and stunt the current years growth. With stunted and retarded growth, lower stored reserves and immature cuttings can be expected relative to unaffected twigs. Lowered stored reserves, as well as immaturity, could reduce a cuttings rooting ability.

The correlation between the percent of rooted cuttings and the time factor might also be due to the apparent slight geographic trend in rooting ability relative to collection sequence, or the relation between initial cutting length (with a north-south difference) and rooting ability. However, since there is no conclusive data concerning the effects of storage on the rooting of shortleaf pine, the possibility exists that a certain period of storage time of the nature used in this study may increase rooting percentages. Studies concerning storage time and methods need to be further examined to determine the exact effects of storage on vegetative material of pine species.

Since source and ortet affects were confounded with row and column percentages of rooting observed on the propagation table, analysis of row and column percentages to determine if any mico-environmental affects on rooting were present would be of little use. However, visual observation of row and column percentages of rooting indicated there were

no pronounced affects on rooting due to micro-environments in the mist-room.

Broad-sense Heritability

Broad-sense heritability was estimated for all of the root characteristics measured. The heritability estimates for the root characteristics of interest are provided in Table VII.

TABLE VII

HERITABILITY ESTIMATES FOR ROOT CHARACTERISTICS

TRAIT	ESTIMATE	STANDARD ERROR
Tap Root Length	.43	$\pm$.047
Total Root Length	.46	$\pm$.046
Root Dry Weight	.40	$\pm$.048
Total Root Number	.41	$\pm$.048
Rooting Ability	.26	$\pm$.022
Root Mass Per Unit Root Length	.26	$\pm$.048

All heritability estimates are generally high and it appears that some reasonable gains can be obtained by selecting within populations for more fibrous or spreading root systems. Development of such propagules should result in the production of individuals which have a greater ability to survive and utilize soil nutrients and moisture. The broad-sense heritability estimate for rooting ability was also high and suggests that rooting ability can be improved through selection and used as a tool in tree improvement.

CHAPTER V

SUMMARY AND CONCLUSIONS

Examination of geographic and clonal variation in rooting ability of vegetative cuttings of shortleaf pine indicated that improvement in rooting ability is possible through individual tree selection and that origin is probably of limited importance. Analysis of the mass per unit length ratio of the roots suggested that there are differences in root structure from one geographic location to the next, which might make origin important in terms of root structure. The importance and/or usefulness of these differences in root structure has yet to be determined. Only through field testing of selected clones can reliable information concerning the effects of these structural differences on survival be quantified.

The large amount of variation observed in the root characteristics from tree to tree and the relatively high estimates of broad-sense heritability suggest that significant improvement in root structure can also be achieved through clonal selection. The ultimate objective of such a selection program would be to increase the survival and growth of the selected clones.

Since rooting ability and structural characteristics of root systems formed undoubtedly affect initiation of growth and field survivability, respectively, studies of the nature of this study, along with field testing of samples of the rooted propagules, are needed to identify easily rootable types with superior root characteristics.

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APPENDIXES

APPENDIX A

LISTING OF RAW DATA ON NUMBER OF ROOTED CUTTINGS

LIST OF ORTETS BY SOURCE SHOWING
NUMBER OF CUTTINGS ROOTED

Source	Ortet no. 1	2	3	4	5	6	7	8	9	10	11	12	13	Total
1	3	0	0	3	4	0	2	0	0	5	0	5	5	27
2	4	0	2	0	3	5	0	0	0	0	3	0	4	21
3	2	0	2	0	3	4	0	2	5	0	2	3	0	23
4	0	3	3	0	0	3	0	0	2	0	0	3	5	19
5	3	0	4	0	0	0	5	0	4	2	5	0	2	25
6	3	0	6	0	0	0	0	0	3	5	0	0	0	17
7	0	8	0	0	0	5	0	5	0	0	3	0	0	21
8	2	5	2	6	4	0	2	5	3	6	0	0	5	41
9	6	5	0	1	0	4	0	4	0	0	8	0	4	32
10	0	0	3	4	0	0	5	0	2	0	0	0	3	17
11	0	8	3	0	6	0	4	0	0	0	0	0	0	21
12	0	0	0	0	0	6	0	0	0	0	4	0	6	16
13	0	4	1	0	2	0	6	0	0	3	1	0	4	21
14	0	4	4	4	0	3	0	0	0	0	9	0	3	27
15	3	5	1	0	3	1	6	7	3	1	1	6	5	42
16	2	4	0	7	3	1	0	6	0	6	0	0	5	34
17	7	2	6	5	4	0	4	5	0	5	0	0	3	41
18	4	0	0	5	0	0	5	0	4	5	3	0	4	30
19	3	0	0	0	8	0	0	0	1	3	5	0	0	20
20	0	4	2	0	3	7	2	0	0	5	0	3	0	26
21	3	2	0	0	0	3	0	4	4	3	6	2	3	30
22	5	3	2	0	0	6	0	7	0	2	0	2	3	30

APPENDIX B

ANALYSIS OF VARIANCE FOR ROOT AND STEM CHARACTERISTICS

ANALYSIS OF VARIABLE OLD GROWTH

VARIABLE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	145.88396	0.24937	0.24993	100.00
LOC	21	11.65135	0.55483	0.00632	2.53
ORTET	127	45.25206	0.35632	0.03999	16.00
CUTN	437	88.98056	0.20362	0.20362	81.47

MEAN	1.467577
STANDARD DEVIATION	0.451239
COEFFICIENT OF VARIATION	0.307472

ANALYSIS OF VARIABLE INITIAL CUTTING LENGTH

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	10143.00143	17.33846	17.55974	100.00
LOC	21	2871.42122	136.73434	4.19818	23.91
ORTET	127	2894.53804	22.79164	3.34545	19.05
CUTN	437	4377.04217	10.01611	10.01611	57.04

MEAN	19.112969
STANDARD DEVIATION	3.164825
COEFFICIENT OF VARIATION	0.165585

ANALYSIS OF VARIABLE INITIAL CUTTING GREEN WEIGHT

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	5589.43038	9.55458	9.59293	100.00
LOC	21	637.28449	30.34688	0.67234	7.01
ORTET	127	1491.12778	11.74116	1.00064	10.43
CUTN	437	3461.01812	7.91995	7.91995	82.56

MEAN	8.480205
STANDARD DEVIATION	2.814241
COEFFICIENT OF VARIATION	0.331860

ANALYSIS OF VARIABLE ROOTED CUTTING DRY WEIGHT

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	250.87401	0.42884	0.43250	100.00
LOC	21	50.86083	2.42194	0.05863	13.56
ORTET	127	96.63626	0.76092	0.13731	31.75
CUTN	437	103.37693	0.23656	0.23656	54.70

MEAN	1.675803
STANDARD DEVIATION	0.486375
COEFFICIENT OF VARIATION	0.290234

ANALYSIS OF VARIABLE TOTAL ROOT LENGTH

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	0.274028D 06	0.468423D 03	0.470260D 03	100.00
LOC	21	31619.25683	1505.67890	9.85385	2.10
ORTET	127	0.136672D 06	0.107616D 04	0.218447D 03	46.45
CUTN	437	0.105736D 06	0.241959D 03	0.241959D 03	51.45

MEAN	16.803413
STANDARD DEVIATION	15.555027
COEFFICIENT OF VARIATION	0.925706

ANALYSIS OF VARIABLE ROOT DRY WEIGHT

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	1.45054	0.00248	0.00249	100.00
LOC	21	0.12909	0.00615	0.00000	0.15
ORTET	127	0.67078	0.00528	0.00099	39.95
CUTN	437	0.85067	0.00149	0.00149	59.89

MEAN	0.046423
STANDARD DEVIATION	0.038587
COEFFICIENT OF VARIATION	0.831205

ANALYSIS OF VARIABLE TAP ROOT LENGTH

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	6055.51565	10.35131	10.66270	100.00
LOC	21	407.56666	19.40794	-0.29659	0.0
ORTET	127	3011.51998	23.71276	4.62968	43.42
CUTN	437	2636.42901	6.03302	6.03302	56.58

MEAN	5.404949
STANDARD DEVIATION	2.456220
COEFFICIENT OF VARIATION	0.454439

ANALYSIS OF VARIABLE TOTAL ROOT NUMBER

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	39089.59898	66.81983	67.05452	100.00
LOC	21	4186.18916	199.34234	1.27737	1.90
ORTET	127	18297.91140	144.07804	27.77829	41.43
CUTN	437	16605.49841	37.99885	37.99885	56.67

MEAN	6.162116
STANDARD DEVIATION	6.164321
COEFFICIENT OF VARIATION	1.000358

ANALYSIS OF VARIABLE ROOT UNIT WEIGHT

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	0.328452D-02	0.561456D-05	0.565581D-05	100.00
LOC	21	0.589603D-03	0.280763D-04	0.671152D-06	11.87
ORTET	127	0.116055D-02	0.913816D-05	0.147351D-05	26.05
CUTN	437	0.153437D-02	0.351115D-05	0.351115D-05	62.08

MEAN	0.003472
STANDARD DEVIATION	0.001874
COEFFICIENT OF VARIATION	0.539614

ANALYSIS OF VARIABLE CUTTING UNIT WEIGHT

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	13.97972	0.02390	0.02398	100.00
LOC	21	1.45395	0.06924	0.00162	6.74
ORTET	127	3.23757	0.02549	0.00111	4.63
CUTN	437	9.28820	0.02125	0.02125	88.63

MEAN	0.448750
STANDARD DEVIATION	0.145789
COEFFICIENT OF VARIATION	0.324878

ANALYSIS OF VARIABLE ROOTED CUTTING STEM DIAMETER

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	585	1.17399	0.00201	0.00201	100.00
LOC	21	0.11493	0.00547	0.00004	2.04
ORTET	127	0.49123	0.00387	0.00067	33.41
CUTN	437	0.56782	0.00130	0.00130	64.55

MEAN	0.175235
STANDARD DEVIATION	0.036047
COEFFICIENT OF VARIATION	0.205704

ANALYSIS OF VARIABLE ROOTED

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	2859	465.93147	0.16297	0.16347	100.00
LOC	21	10.28531	0.48978	-0.00039	0.0
ORTET	264	142.64615	0.54033	0.04187	25.61
CUTN	2574	313.00000	0.12160	0.12160	74.39

ANALYSIS OF VARIABLE ALIVE

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	2859	336.09056	0.11756	0.11765	100.00
LOC	21	7.89825	0.37611	0.00160	1.36
ORTET	264	44.49231	0.16853	0.00583	4.96
CUTN	2574	283.70000	0.11022	0.11022	93.69

ANALYSIS OF VARIABLE CALLUSED

VARIANCE SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	VARIANCE COMPONENT	PERCENT
TOTAL	2859	494.56783	0.17299	0.17326	100.00
LOC	21	20.21399	0.96257	0.00545	3.15
ORTET	264	66.95385	0.25361	0.00953	5.50
CUTN	2574	407.40000	0.15828	0.15828	91.35

VITA

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